

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

BEST AVAILABLE COPY

| | | |
|--|-----------|--|
| (51) International Patent Classification ⁵ : A61K 31/74, 31/725, 37/547 A01N 37/18 | A1 | (11) International Publication Number: WO 92/00747 (43) International Publication Date: 23 January 1992 (23.01.92) |
| (21) International Application Number: PCT/US91/02868 (22) International Filing Date: 2 May 1991 (02.05.91) (30) Priority data: 551,924 12 July 1990 (12.07.90) US 662,452 28 February 1991 (28.02.91) US (71) Applicant: STERILIZATION TECHNICAL SERVICES, INC. [US/US]; 7500 West Henrietta Road, Rush, NY 14543 (US). (72) Inventors: WHITBOURNE, Richard, James ; 1 Gateway Road, Fairport, NY 14450 (US). MANGAN, Margaret, Anne ; 509 Highland Avenue, Rochester, NY 14620 (US). (74) Agent: MARJAMA, Owen, D.; Marjama & Pincelli, 488 White Spruce Blvd., Rochester, NY 14623 (US). | | (81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i> |
| (54) Title: ANTI-THROMBOGENIC AND/OR ANTI-MICROBIAL COMPOSITION | | |
| (57) Abstract Anti-thrombogenic, and/or anti-microbial and/or pharmaceutical compositions containing heparin and/or antibiotics and/or other pharmaceutical agents which may be reacted with quaternary ammonium components or other ionic surfactants and bound with water-insoluble polymers are disclosed. Such compositions may also contain additional quaternary ammonium compounds or other ionic surfactants not reacted with heparin and may also contain quaternary ammonium compound(s) or other ionic surfactants reacted with antibiotics or other ionic or nonionic pharmaceutical agents. | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | ES | Spain | MG | Madagascar |
| AU | Australia | FI | Finland | ML | Mali |
| BB | Barbados | FR | France | MN | Mongolia |
| BE | Belgium | GA | Gabon | MR | Mauritania |
| BF | Burkina Faso | GB | United Kingdom | MW | Malawi |
| BG | Bulgaria | GN | Guinea | NL | Netherlands |
| BJ | Benin | GR | Greece | NO | Norway |
| BR | Brazil | HU | Hungary | PL | Poland |
| CA | Canada | IT | Italy | RO | Romania |
| CF | Central African Republic | JP | Japan | SD | Sudan |
| CG | Congo | KP | Democratic People's Republic of Korea | SE | Sweden |
| CH | Switzerland | KR | Republic of Korea | SN | Senegal |
| CI | Côte d'Ivoire | LI | Liechtenstein | SU | Soviet Union |
| CM | Cameroon | LK | Sri Lanka | TD | Chad |
| CS | Czechoslovakia | LU | Luxembourg | TC | Togo |
| DE | Germany | MC | Monaco | US | United States of America |
| DK | Denmark | | | | |

ANTI-THROMBOGENIC, AND/OR ANTI-MICROBIAL COMPOSITIONBACKGROUND OF THE INVENTION

This application is a Continuation-in-Part of U.S. Serial No. 07/551,924 filed July 12, 1990, entitled ANTI-THROMBOGENIC, AND/OR ANTI-MICROBIAL COMPOSITION which is a Continuation-in-Part application, U.S. Serial No. 07/430,340, filed November 2, 1989, entitled ANTI-THROMBOGENIC, ANTI-MICROBIAL COMPOSITIONS CONTAINING HEPARIN.

Many kinds of polymer compositions have been used in the field of medical supplies. These compositions have not always exhibited anti-thrombogenic, anti-microbial, or other biocompatible characteristics when used in prosthetic and therapeutic apparatuses for handling or being in contact with blood or blood components or other bioresponse under conditions where clotting would tend to occur, such as artificial blood vessels, catheters, artificial hearts, fluid drainage, suction/aspiration and artificial kidneys.

When blood is brought in contact with metal, glass, plastic or other similar surfaces, it tends to clot in a short time unless certain precautions are taken. One common precaution currently in widespread use is the treatment of the surface with heparin or with heparin reacted with quaternary ammonium compounds. Such heparin compounds are known to have anti-coagulant effects when in contact with blood. The presence of the aforementioned heparin compounds on the surface imparts anti-thrombogenic characteristics. However, previously known heparinization or compositions have not been adequate because of the short time of anti-thrombogenic activity, at most a few days in vivo (I. S. Hersch, et al, J. Biomed., Mater. Res. Symposium I, 99-104 (1971); K. Amplatz, "A Simple Non-Thrombogenic Coating", Invest. Radiology, July, August, 1971, Vol. 6 or because the anti-thrombogenic characteristic was reduced to a very low level in order to make it resistant to removal by

reacting it with quaternary ammonium polymers (U.S. Patent 3,844,989).

It is therefore, an object of the present invention to provide novel anti-thrombogenic/polymer/heparin compound compositions or mixtures which prevent blood clotting for a relatively long period of time (over one month), and which have the same high degree of anti-thrombogenic characteristics as the non-polymerized heparin-quaternary ammonium compounds, and thus provide excellent properties for use as medical materials for coatings on artificial blood vessels, catheters, artificial hearts, artificial kidneys, etc.

Another object of the present invention is to provide novel anti-microbial surfaces which contain antibiotic agents which are entrained in the surface in such a way as to be gradually released in vivo to provide effective anti-microbial action over a longer time than was previously possible when using these agents. Typical agents useful in this embodiment of the invention include penicillins, cephalosporins, aminoglycosides, quinolones, sulfonamides, tetracyclines, etc. While effective anti-microbial agent concentrations are achieved near the coated device surface, low systemic levels result, unlike where systemic antibiotic administration is required to combat infections caused by an implanted device.

SUMMARY OF THE INVENTION

The anti-thrombogenic, anti-microbial, pharmaceutical agent compositions (mixtures) of this invention comprise heparin-quaternary ammonium compounds and/or other ionic pharmaceutical agent-ionic surfactant compounds mixed with water-insoluble polymers. Pharmaceutical agents that are not reacted with ionic surfactants may also be used, providing that they have the appropriate solubility profile namely that they are soluble in organic solvents. They may also contain some hydrophilic polymers, but the mixture would still be water-insoluble after coating and drying. The water-insoluble

polymers of this invention range from hydrophobic polymers to ones that are fairly hydrophilic, but are nevertheless essentially water-insoluble after being coated on a substrate and dried. A single polymer or mixture(s) of different polymers may be used to accomplish the invention. The heparin-quaternary ammonium compound may be mixed in a solution with the water-insoluble polymer, or it may be coated on top of a coating of the water-insoluble polymer(s), which is applied to the surface beforehand. In the latter case, a solvent must be added that is a mutual solvent for both the heparin-quaternary ammonium compound and the water-insoluble polymer(s) so that some mixing occurs between the two layers. In still another case, it is possible to coat the heparin-quaternary ammonium compound directly on the water-insoluble plastic surface, and incorporate a mutual solvent for both the plastic surface and the heparin-quaternary ammonium compound, so that some mixing occurs between the plastic surface and the heparin-quaternary ammonium compound.

Various combinations of these three systems would be obvious to one skilled in the art. The mixtures of the water-insoluble polymer(s) and heparin-quaternary ammonium compounds of this invention are substantially more resistant to removal or deactivation in human and animal body fluids such as blood or plasma than the heparin-quaternary ammonium compounds by themselves.

Typical examples of polymers suitable for use with the present invention are as follows: Water insoluble cellulose esters such as cellulose acetate, cellulose acetate butyrate, cellulose acetate propionate, and cellulose nitrate; polyurethane resins including polyether and polyester grades. Exemplary of the polyurethane is the reaction product of 2,4-tolylene diisocyanate and position isomers thereof, 4,4'-diphenylmethane diisocyanate and position isomers thereof, polymethylenepolyphenyl isocyanate, or 1,5-naphthylene diisocyanate with 1,2-polypropylene glycol, polytetramethylene

ether glycol, 1,4-butanediol, 1,4-butylene glycol, 1,3-butylene glycol, poly(1,4-oxybutylene)glycol, caprolactone, adipic acid esters, phthalic anhydride, ethylene glycol, 1,3-butylene glycol, 1,4-butylene glycol or diethylene glycol. Acrylic polymers such as ethyl and methyl acrylate and methacrylate; condensation polymers such as those produced by sulfonamides such as toluenesulfonamide and aldehydes such as formaldehyde; and isocyanate compounds. Exemplary of the isocyanate compounds are polymethylenepolyphenyl isocyanate, 4,4'-diphenylmethane diisocyanate and position isomers thereof, 2,4-tolylene diisocyanate and position isomers thereof, 3,4-dichlorophenyl diisocyanate and isoferrone isocyanate. Adducts or prepolymers of isocyanates and polyols such as the adduct of trimethylolpropane and diphenylmethane diisocyanate or tolylene diisocyanate are suitable. For further examples of polyisocyanates, see "Encyclopedia of Polymer Science and Technology", H. F. Mark, N. G. Gaylord and N. M. Bikales (eds.) (1969) incorporated herein by reference.

Typical quaternary ammonium compounds that can be reacted with heparin for use in this invention include benzalkonium chloride, tridodecylmethylammonium chloride, cetylpyridinium chloride, benzyldimethylstearylammmonium chloride, benzylcetyl-dimethylammonium chloride, etc.

DETAILED DESCRIPTION OF THE INVENTION

The compositions of the present invention are first dissolved in solvent mixtures that are co-solvents for the mixtures of non-volatile components and which allow compatible homogenous films of the components to be cast. Such films when dried will typically appear as a clear film or films of very slight turbidity indicating that the non-volatile components have been deposited in a substantially homogenous manner. Typical solvents comprise alcohols, ketones, esters, aromatics, pyrrolidones,

carboxylic acids, amides, and other organic solvents used alone or in appropriate mixtures as required, and which bring about the basic compatibility of the non-volatile components to be expressed. Typical surfaces which can be coated include plastic, metal and glass.

The heparin-quaternary ammonium compounds may be prepared in the conventional manner by any known prior art technique. For example, a heparin-benzalkonium chloride compound can be prepared by mixing approximately equal volumes of a 10% (by wt.) aqueous solution of sodium heparin with an approximately 17% (by wt.) solution of benzalkonium chloride (i.e., Zephiran from Winthrop-Breon Laboratories), and then washing the residual sodium chloride out with distilled or deionized water. Such preparations are disclosed in "A Simple Non-Thrombogenic Coating", K. Amplatz, Invest., Radiology, July, August, 1971, Vol. 6, which is incorporated herein by reference. It should be understood, however, that the invention is not limited to the heparin-quaternary ammonium compounds cited in the above reference.

In most cases, all the components are incorporated into a single solution so that the surface treatment can be accomplished with a single application. However, the treatment can also be applied in two steps. For example, the water-insoluble polymer(s) can be applied in one application and the heparin-quaternary ammonium compound can be applied to the water-insoluble polymer. Some mutual solvent(s) for the water-insoluble polymer and heparin-quaternary ammonium compound that makes two components compatible should be included in the overcoat application to accomplish the objective of the invention. For example, dimethylacetamide (DMA) effectively accomplishes this objective as shown in Example 1. A variant on this approach would involve application of the water-insoluble polymer(s) followed by application of a solution containing some water-insoluble polymers and some heparin-quaternary ammonium

compound. Some heparin-quaternary ammonium compounds may also be added to the first application. Typical concentrations of heparin-quaternary ammonium compound in the coating solutions range from about 0.1% to 20% by weight. Preferred concentrations range from 0.5% up to 4%. Use of higher concentrations of heparin-quaternary ammonium compounds in the solutions does not enhance performance and is therefore not very useful or desired. Lower concentrations than those disclosed above reduce the anti-thrombogenicity of the layers.

Typical concentrations of the water-insoluble polymers in the coating solution range from about .01% to 20% by weight. Preferred concentrations range from about .2% to 3%. Higher concentrations tend to mask the anti-thrombogenic characteristics of the layers. Lower concentrations tend to allow the layer to be extracted more easily. The composition of the final coating may have the heparin quaternary compound present in a concentration of about 0.5 to 99.5 percent by weight with the balance of the composition comprising essentially the water-insoluble polymer.

ANTI-THROMBOGENICITY TEST

The following in vitro test was used to evaluate anti-thrombogenicity: 10mm x 75mm glass test tubes were charged with 0.5 gm of reconstituted human plasma which had been kept refrigerated since collection. The test tubes were equilibrated in a 37°C incubator for 10-30 minutes. Next, 0.1 g of 0.10 M CaCl_2 was added, and the test tube was manually swirled to achieve complete mixing. Immediately after swirling, 4-1/2" long sections of 7 French tubing (either coated with one of the anti-thrombogenic systems of the present invention, or uncoated controls) were dropped into the plasma in each tube, taking care to ensure that the sample pieces were completely immersed in the plasma. The tubes were maintained in the 37°C incubator and were

checked for clotting at one minute intervals by removing them from the incubator and tilting them. Before clotting, the liquid flows in the test tube, but it gels and does not flow once it has clotted. Typical clotting times for plasma containing untreated polyurethane tubing range from six minutes to 15 minutes. Samples made according to this invention prevent clotting in this test. It was found that if the plasma did not clot after standing overnight, it would usually not clot for up to four weeks. Therefore, tests were usually discontinued if they had not clotted after standing overnight. Typical samples prepared by this invention did not clot when tested before plasma extraction, and retained their anti-clotting activity after 28 or more days of extraction in plasma. Devices coated with heparin-benzalkonium chloride or heparin-tridodecylmethylammonium chloride do not clot when tested before extraction in plasma, but lose their anti-thrombogenicity after plasma extraction of two hours or less. Heparinized quaternary polymers (HQP), such as those prepared according to U.S. Patent 3,844,989 and used on catheters marked under the trademark ANTHRON by Toray Medical Co. Ltd., show only slight anti-thrombogenicity. For example, when tested against heparin-benzalkonium chloride (HBAC), the HBAC sample prevented clotting of the plasma overnight, while the control clotted in five minutes and the HQP sample clotted in seven minutes before plasma extraction, and showed no improvement in anti-thrombogenicity compared to the untreated polyurethane control after 12 hours of plasma extraction.

The following examples are intended to illustrate various preferred embodiments of the present invention.

Example 1

Polyurethane 7 French tubing was coated with a solution containing the following ingredients and dried for 20 minutes at 65°C.

| | |
|---------------------------|--------|
| Polyvinylpyrrolidone | .006 g |
| Isopropanol | 1.0 g |
| Nitrocellulose | 1.6 g |
| Ethylacetate | 1.2 g |
| Rosin ester | .5 g |
| Butylacetate | 4.8 g |
| Dimethylacetamide | 1.5 g |
| Ethyl-3-ethoxy propionate | 6.1 g |

The tubing was then overcoated with a solution containing the following ingredients and then dried for 20 minutes at 65°C.

| | |
|-------------------------------|--------|
| Isopropanol | 9.85 g |
| Dimethylacetamide | 1.00 g |
| Heparin-benzalkonium chloride | .15 g |

This sample was compared to a sample of polyurethane tubing which was coated with heparin-benzalkonium chloride (1.8% w/v in isopropanol) as follows. The samples were dipped in a Gentian Violet dye solution and then rinsed in hot running water. The sample coated with heparin-benzalkonium chloride (HBAC) in isopropanol lost most of the surface dye stain in less than 20 seconds, indicating that most of the HBAC had been washed off. The sample of the present invention that had the nitrocellulose undercoat and contained DMA in the HBAC overcoat, retained the dye stain much longer indicating that it is much more resistant to removal.

Example 2

Polyurethane 7 French tubing was coated with a solution consisting of:

| | |
|-------------------------------|--------|
| Methylethylketone | 5.0 g |
| Heparin-benzalkonium chloride | 0.33 g |
| Isopropanol | 3.7 g |
| Ethyl-3-ethoxy propionate | .6 g |
| Butyl acetate | .5 g |
| 1/2 sec. nitrocellulose | .16 g |
| Ethyl acetate | .1 g |
| Rosin ester | .05 g |

The samples were dried at 75°C for 30 minutes. Samples were then extracted in human plasma at 37°C for 7, 10, 21, or 28 days and then tested for anti-clotting properties. The following results were obtained.

| <u>Sample</u> | <u>Clotting time</u> |
|--|----------------------|
| Uncoated control | 12 minutes |
| Above sample, without extraction in plasma | Did Not Clot |
| Above sample, after 7 days extraction in plasma | Did Not Clot |
| Above sample, after 10 days extraction in plasma | Did Not Clot |
| Above sample, after 21 days extraction in plasma | 24 minutes |
| Above sample, after 28 days extraction in plasma | 20 minutes |

The above results show that the samples are still exhibiting effective anti-clotting activity on the device surface where it is most needed and that clots are unlikely to form on the treated surfaces, even after 28 days of extraction. This level of anti-clotting activity is stronger even after 28 days of plasma extraction than the anti-clotting levels achieved under these test conditions with surfaces treated according to the compositions taught by U.S. Patent 3,844,989.

Example 3

The following solution was coated on polyurethane 7 French tubing and dried at 75°C for 20 minutes.

| | |
|--|-------|
| Methylethylketone | 5 g |
| 8.3% heparin-benzalkonium chloride in isopropanol | 5 g |
| Cellulose Acetate Butyrate - 3A solution* | 1.5 g |

*3A solution

| | |
|--|--------|
| Ethyl-3-ethoxy propionate | 30.3 g |
| Butylacetate | 24.2 g |
| Ethyl acetate | 6.1 g |
| Rosin ester | 1.5 g |
| Isopropanol | 3.5 g |
| 1/2 sec. Cellulose acetate butyrate | 8.0 g |

Coated samples were tested for anti-clotting activity, and also for resistance to removal by dyeing with Gentian Violet dye and then rinsing with hot running water. The sample was compared to a coating of heparin-benzalkonium chloride without any cellulose ester polymer additive.

Results: The sample did not clot in the clotting test. In the hot water rinse test, the heparin-benzalkonium chloride coating without cellulose resin was completely removed in a few seconds. Hot water rinsing did not remove the above coating which contained cellulose acetate butyrate polymer.

Example 4

Polyurethane 7 French tubing was coated as in Example 3 except that cellulose acetate butyrate was replaced with cellulose acetate propionate. The sample was tested for anti-clotting activity and resistance to removal in hot water. Results were comparable to those with Example 3.

Example 5

Polyurethane 7 French tubing was coated with the following solution and dried at 80°C for 20 minutes.

| | |
|--|-----|
| Methylethylketone | 5 g |
| 8.3% heparin-benzalkonium chloride in isopropanol | 4 g |
| Cellulose acetate propionate 5A solution* | 2 g |

*5A solution

| | |
|--------------------------------------|--------|
| Ethyl-3-ethoxy propionate | 30.3 g |
| Butylacetate | 24.2 g |
| Ethylacetate | 6.1 g |
| Rosin ester | 2.5 g |
| Isopropanol | 3.5 g |
| 1/2 sec.cellulose acetate propionate | 8.0 g |

The coated sample was extracted in plasma at 37°C for four hours and tested for anti-microbial activity by pressing it into gelled Difco Plate Agar which was spiked with Staphylococcus epidermidis (ATCC 12228) and then incubated overnight at 32-35°C. A sample of polyurethane tubing that was coated with heparin-benzalkonium chloride without cellulose polymer was extracted in plasma at 37°C for four hours for comparison. The sample which contained cellulose acetate propionate (CAP) polymer showed a significant zone of inhibition while the sample made without CAP resin showed no zone of inhibition, demonstrating that the incorporation of cellulose ester polymer effectively increases resistance to removal of the coating when extracted in human plasma.

Example 6

Example 5 was repeated, except that the solution contained 1.5 gm of 10.7% (wt. %) nitrocellulose solution in place of the 2.0 grams of 10.7% (wt. %) CAP solution. Samples of polyurethane tubing coated with this solution were extracted in plasma at 37°C for four hours or 18 hours. They were then tested for anti-microbial activity using the same zone of inhibition test as used in Example 5. The tests showed zones of inhibition after both extraction intervals. The sample extracted for four hours has a larger zone of inhibition than the sample that was extracted for 18 hours.

Example 7

The following solution was coated on polyurethane 7 French tubing and dried at 80°C for 20 minutes. A control was made by coating a sample of the tubing with a 5% w/v solution of Tridodecylmethylammonium chloride (TDMAC).

| | |
|---------------------------------------|-------|
| Methylethylketone | 5 g |
| 8.3% heparin-benzalkonium chloride in | |
| isopropanol | 4 g |
| 7A solution* | 1.5 g |

*7A solution

| | |
|---------------------------|--------|
| Ethyl-3-ethoxy propionate | 30.3 g |
| Butylacetate | 24.2 g |
| Ethyl acetate | 6.1 g |
| Rosin ester | 2.5 g |
| Isopropanol | 3.5 g |
| 1/2 sec. Nitrocellulose | 8.0 g |

Both samples were then immersed for 30 minutes in a 5% aqueous solution of penicillin G and then air dried overnight. The coated samples were then extracted for 18 hours in human plasma at 37°C. They were removed from the plasma, rinsed in running deionized water and then tested for anti-microbial activity as in Example 5. The sample containing nitrocellulose showed a strong zone of inhibition while the sample without nitrocellulose showed no zone of inhibition.

Example 8

Example 7 was repeated, except that TDMAC was added to the coating solutions as follows:

Example 8 .025 gm TDMAC added

Example 8A .075 gm TDMAC added

Both samples showed a strong zone of inhibition after the 18 hours plasma extraction and appeared to be substantially comparable to Example 7.

Example 9

Polyurethane 7 French tubing was coated with the following solution.

Heparin tridodecylmethylammonium chloride 0.2 g

Isopropanol 2.6 g

Methylethylketone 2.5 g

7A Solution 0.7 g

This coated sample was tested for clotting and did not clot. It was very resistant to removal in hot running water.

Example 10

Polyurethane 7 French tubing was coated with a solution containing the following ingredients and dried at ambient temperature for 60 minutes:

| | |
|-----------------------------------|--------|
| Methylethylketone | 5.3 g |
| Heparin-benzalkonium chloride | 0.31 g |
| Isopropanol | 3.4 g |
| Acrylic resin | 0.2 g |
| Rosin ester | 0.2 g |
| Tridodecylmethylammonium chloride | 0.4 g |
| Xylene | 0.14 g |
| Butanol | 0.05 g |

Samples were then extracted in plasma at 37°C for 4, 24 and 120 hours and compared to uncoated polyurethane tubing for anti-clotting activity. The results were as follows:

| <u>Sample</u> | <u>Clotting Time</u> |
|--|----------------------|
| Uncoated control | 9 minutes |
| Above sample, without extraction in plasma | Did Not Clot |
| Above sample, after 4 hours extraction in plasma | Did Not Clot |
| Above sample, after 24 hours extraction in plasma | Did Not Clot |
| Above sample, after 120 hours extraction in plasma | Did Not Clot |

The above coated sample was resistant to removal by hot running water.

Example 11

Polyurethane 7 French tubing was coated with a solution containing the following ingredients and dried 15 minutes at 75°C:

| | |
|-------------------------------|--------|
| Methylethylketone | 5.6 g |
| Heparin-benzalkonium chloride | 0.33 g |
| Isopropanol | 3.5 g |
| Polyurethane resin | 0.24 g |
| Polyisocyanate resin | 0.19 g |
| Ethyl acetate | 0.19 g |

Samples were extracted in plasma at 37°C for 72 hours and then tested for anti-clotting properties. A sample of polyurethane tubing which was coated with heparin-benzalkonium chloride (1.8% w/v in isopropanol) was also extracted in plasma at 37°C for 72 hours for comparison. The following results were obtained:

| <u>Sample</u> | <u>Clotting Time</u> |
|--|----------------------|
| Uncoated control | 13 minutes |
| Above sample, after 72 hours extraction in plasma | Did Not Clot |
| Sample coated with heparin-benzalkonium chloride in isopropanol, after 72 hours extraction in plasma | 7 minutes |
| The above coating was also resistant to removal by hot running water. | |

Example 12

Polyurethane 7 French tubing was coated with a solution containing the following ingredients and dried for 20 minutes at 70°C.

| | |
|-------------------------------|--------|
| Methylethylketone | 5.9 g |
| Heparin-benzalkonium chloride | 0.32 g |
| Isopropanol | 3.5 g |
| Polyurethane resin | 0.14 g |
| Polyisocyanate resin | 0.07 g |
| Ethylacetate | 0.07 g |

Samples were then extracted in human plasma at 37°C for 3, 24, and 48 hours and then tested for anti-clotting properties. The following results were obtained:

| <u>Sample</u> | <u>Clotting Time</u> |
|---|----------------------|
| Uncoated control | 8 minutes |
| Above sample, after 3 hours extraction in plasma | Did Not Clot |
| Above sample, after 24 hours extraction in plasma | Did Not Clot |
| Above sample, after 48 hours extraction in plasma | 9 minutes |

Example 13

Polyurethane 7 French tubing was coated with a solution containing the following ingredients and dried for 20 minutes at 70°C.

| | |
|-------------------------------|--------|
| Methylethylketone | 6.1 g |
| Heparin-benzalkonium chloride | 0.32 g |
| Isopropanol | 3.5 g |
| Polyurethane resin | 0.07 g |
| Polyisocyanate resin | 0.04 g |
| Ethylacetate | 0.04 g |

Coated tubing was then extracted in plasma for 3 and 24 hours and then tested for anti-clotting behavior. The following results were obtained:

| <u>Sample</u> | <u>Clotting Time</u> |
|---|----------------------|
| Uncoated control | 8 minutes |
| Above sample, after 3 hours extraction in plasma | Did Not Clot |
| Above sample, after 24 hours extraction in plasma | 9 minutes |

Example 14

Polyurethane 7 French tubing was coated with a solution containing the following ingredients and dried for 20 hours at 55°C.

| | |
|--|--------|
| Heparin tridodecylmethyammonium chloride | 0.32 g |
| Dimethylacetamide | 6.2 g |
| Toluene | 2.0 g |
| Petroleum ether | 1.5 g |

The coated tubing was extracted in human plasma at 37°C for 1, 2, 3 and 6 days and then tested for anti-clotting properties.

| <u>Sample</u> | <u>Clotting Time</u> |
|---|----------------------|
| Uncoated sample | 10 minutes |
| Above sample, after 1 day extraction in plasma | Did Not Clot |
| Above sample, after 2 days extraction in plasma | Did Not Clot |
| Above sample, after 3 days extraction in plasma | Did Not Clot |
| Above sample, after 6 days extraction in plasma | Did not Clot |

The preceding examples, together with controls, show clearly that heparin-quaternary ammonium compounds and other pharmaceutical agents that are not polymeric can be made more resistant to removal or deactivation in various body fluids such as whole blood or plasma (including human) by mixing with appropriate water-insoluble polymers. Coatings made from normal heparin-quaternary ammonium compounds by themselves using solvents that do not cause mixing with the substrate, such as heparin-benzalkonium chloride, or heparin

tridodecylmethyammonium chloride show little anti-thrombogenicity after soaking in human plasma for only a few hours. The heparin-TDMAC compound continues to show anti-thrombogenicity somewhat longer than the benzalkonium chloride compound, but both exhibit almost no anti-thrombogenicity after soaking in human plasma for a few hours. The incorporation of water-insoluble polymers according to the present invention, and as shown in the examples, greatly extends the time for which coating samples can be soaked in human plasma and still show substantially levels of anti-thrombogenicity. For instance, some samples were found to show anti-thrombogenicity even after soaking in human plasma for 28 days.

On the other hand, when quaternary ammonium polymers are reacted with heparin, the coating remains on the surface even after long periods of soaking in body fluids such as human plasma, but the anti-thrombogenicity is not as strong either before soaking or after soaking for up to 28 days in human plasma, as in the samples made according to this invention. It is further noted that by water-insoluble polymers we are implying that they are water-insoluble after a film is cast and dried, and include water-insoluble polymers that may be hydrophilic, but nevertheless cause the heparin-quaternary ammonium compounds to remain anti-thrombogenic after prolonged soaking in body fluids.

In a further embodiment of the present invention, it has been found that it is possible to react an antibiotic or other pharmaceutical agent such as penicillin, ticarcillin, cefotoxin, cephalosporins, oxacillin, and carbonicillin that contains a positive inorganic ion such as sodium with a quaternary ammonium compound such as benzalkonium chloride or (TDMAC) to produce an ionic antibiotic that is soluble in organic solvents and is miscible with hydrophobic water insoluble polymers. In this embodiment, the resulting polymer mixture would not contain an anti-thrombogenic agent such as heparin. It is also possible to react other antibiotics or pharmaceutical agents that contain a

negative ion such as chloride with surfactants that contain a negative organic ion such as sodium laurylsulfate to again convert a water soluble antimicrobial agent or other pharmaceutical agent into one that is soluble in organic solvents and miscible with hydrophobic water insoluble polymers. It is also possible to incorporate pharmaceutical agents without reaction with ionic surfactants if the pharmaceutical agent has low water solubility and is soluble in organic solvents and miscible with the hydrophilic water insoluble polymers of the present invention. When these organic solvent soluble agents are mixed with polymers of this invention, they can be rendered much more resistant to removal in plasma from the surface of an article coated with them than if they are coated on the surface without the polymer.

By using antibiotics or other pharmaceutical agents that are, soluble in organic solvents, or by making the antibiotic or other pharmaceutical agent soluble in organic solvents and miscible with the water insoluble polymers of this invention, it makes it possible to incorporate useful pharmaceuticals such as antibiotics onto medical devices at the time of manufacture. The pharmaceuticals are available at the surface of the device in efficacious concentrations, over a useful period such as several days to weeks. At the same time, while the pharmaceuticals are present in useful concentrations where they are wanted on the device surface, they are not present in high concentration systematically so that typical side effects normally associated with various pharmaceuticals are unlikely.

The polymer can be mixed with the pharmaceutical agent and then coated, or the polymer or agent can be coated first and then overcoated with the other agent. The single coating of a mixture of polymer and pharmaceutical agent would normally be preferred because it would involve only a single coating and because the ratio of pharmaceutical agent to polymer can be controlled more precisely. Such mixtures of pharmaceutical agents and polymers

would not be anti-thrombogenic unless they also contained an anti-thrombogenic agent such as heparin. However, the coatings do show strongly the desired effect of the incorporated pharmaceutical agent such as anti-microbial activity. The presence of certain polymers also has the added benefit of enhancing the stability of the pharmaceutical agent to a sterilization process such as exposure to ethylene oxide.

The antibiotic-surfactant compound or other pharmaceutical agent is present in a concentration of about 0.5% to 99.5% by weight with the balance comprising the water-insoluble polymer. The concentration of the water-insoluble polymer is typically about 0.01% to 40% by weight and the concentration of antimicrobial-surfactant compound is about 0.01% to 40% by weight of the coating solution.

The following example demonstrates how the system works.

In Examples 15-18, ¹⁴C-penicillin G sodium salt was reacted with tridodecylmethyammonium chloride (TDMAC) using procedures similar to those previously described in the Background Of The Invention, see A. Amplatz, "A Simple Non-Thrombogenic Coating", Invest. Radiology, July, August, 1971, Vol. 6, which is incorporated herein by reference.

A typical method of preparation for the pharmaceutical agent-TDMAC compounds of the present invention is as follows:

Seventeen grams TDMAC is dissolved in 60 ml isopropanol, and diluted with 40 ml distilled water. Next, dissolve 10 grams of the sodium salt of the pharmaceutical agent (SPA) in 100 ml distilled water. Mix equal volumes of both liquids and shake vigorously for ten or so seconds to ensure complete mixing and reaction.

Next, vacuum filter over filter paper, collect the compound off the paper, and place in a centrifuge jar with 1 volume of water, shake for 30 minutes, and vacuum filter again on filter paper. The wash is repeated twice more. The SPA-TDMAC is dried in an oven at 60°C.

Using this basic procedure, it is obvious to one skilled in the art that organic salts can be made from many or most ionic pharmaceutical agents by mixing them together with an appropriate ionic surfactant, and washing out the water-soluble salt residue with water. These compounds are soluble in organic solvents and typically have very low solubility constants so that when mixed with the polymers of this invention, constant and efficacious concentrations of the pharmaceutical agent (5) will be available on the coated surface in vivo over an extended period.

The resultant ^{14}C -penicillin-TDMAC prepared by the above method is soluble in various organic solvents and has extremely low water solubility but is still ionic. The ^{14}C -penicillin-TDMAC was then mixed with selected polymers and coated on both silicone and polyurethane tubing. The coatings were then extracted in plasma for one day or 5 days and compared to non-extracted samples by scintillation counting to determine how much penicillin remained on the surface in the coating after extraction. Some samples were exposed to an ethylene oxide sterilization cycle and tested by zone of inhibition, to show whether the polymer improved the resistance of the antibiotic to degradation when exposed to ethylene oxide.

Example 15

The ^{14}C -penicillin-TDMAC was mixed with cellulose nitrate dissolved in a solvent mixture containing ethanol, isopropanol, ethylacetate and toluene. The solution has the following composition:

| | |
|-----------------------------------|--------|
| Nitrocellulose | 1.8 g |
| Isopropanol | .8 g |
| Toluene | 24.3 g |
| Ethyl acetate | 5.1 g |
| Camphor | .5 g |
| Dibutylphthalate | .7 g |
| ^{14}C -penicillin-TDMAC | 2.0 g |

The solution was coated on both silicone and polyurethane tubing and dried. Some coated samples were then extracted in plasma for 24 hours or five days. After plasma extraction, the samples were measured by scintillation counting and were compared to unextracted samples to show how much ^{14}C -penicillin-TDMAC remained. The following results were obtained.

| | <u>Not Extracted</u> | <u>After 24 hrs. Extraction</u> | <u>After Five Days Extraction</u> |
|---------------------|------------------------------|-------------------------------------|---------------------------------------|
| Silicone tubing | 48 $\mu\text{g}/\text{cm}^2$ | 30 $\mu\text{g}/\text{cm}^2$ | 6 $\mu\text{g}/\text{cm}^2$ |
| Polyurethane tubing | 36 $\mu\text{g}/\text{cm}^2$ | 43 $\mu\text{g}/\text{cm}^2$ | 38 $\mu\text{g}/\text{cm}^2$ |

When the ^{14}C -penicillin-TDMAC was coated without polymer, it was removed from the tubing surface in a few hours. These results clearly show how incorporation of this polymer into the coating dramatically extends the elution time from the surface when extract in plasma.

Example 16

Example 15 was repeated using Silastic silicone resin in 1,1,1,-Trichloroethane in place of the nitrocellulose solution. The solution has the following composition:

| | |
|----------------------------------|--------|
| Silastic Polymer | 1.3 g |
| 1,1,1-Trichloroethane | 28.7 g |
| Toluene | 8.0 g |
| ¹⁴ C-penicillin-TDMAC | 2.0 g |

This sample was tested for resistance to extraction in plasma, and for resistance to degradation by ethylene oxide sterilization.

Results:

| | <u>Not Extracted</u> | <u>After 24 hrs. Extraction</u> | <u>After Five Days Extraction</u> |
|---------------------|----------------------------|-------------------------------------|---------------------------------------|
| Silicone tubing | 68 μ g/cm ² | 33 μ g/cm ² | 27 μ g/cm ² |
| Polyurethane tubing | 18 μ g/cm ² | 8 μ g/cm ² | 6 μ g/cm ² |

These results show that incorporation of Silastic β resin into the coating extends the elution time of the antibiotic in plasma to several days compared to a few hours without the resins.

After exposure to a typical ethylene oxide sterilization (ETO) cycle, the samples were tested by classic zone of inhibition testing. This was done by placing a sample (sterilized or non-sterilized) onto a layer of agar containing bacteria and then incubated. The results are reported as the size in mm of the clear zone surrounding the coated article which results from the antimicrobial activity of active ¹⁴C-penicillin-TDMAC.

| | <u>Before ETO Exposure</u> | <u>After ETO Exposure</u> |
|-----------------|----------------------------|---------------------------|
| With SilasticB | 11 | 10 |
| Without polymer | 26 | 0 |

This result clearly demonstrates how incorporation of SilasticB polymer into the ¹⁴C-penicillin-TDMAC coating greatly increases the resistance of the antibiotic to degradation caused by exposure to ethylene oxide.

Example 17

Example 15 was repeated using polyvinylbutyral (PVB) polymer in toluene in place of the nitrocellulose solution. The solution has the following composition:

| | |
|----------------------------------|--------|
| Polyvinylbutryral | 1.5 g |
| Toluene | 31.5 g |
| ¹⁴ C-penicillin-TDMAC | 2.0 g |

This was coated on silicone and polyurethane tubings, dried, and then tested for resistance to extraction in plasma, and resistance to degradation during an ethylene oxide sterilization by zone of inhibition. The following results were obtained.

| | <u>Not Extracted</u> | <u>After 24 hrs. Extraction</u> | <u>After Five Days Extraction</u> |
|---------------------|----------------------------|-------------------------------------|---------------------------------------|
| Silicone tubing | 18 μ g/cm ² | 18 μ g/cm ² | 16 μ g/cm ² |
| Polyurethane tubing | 12 μ g/cm ² | 13 μ g/cm ² | 10 μ g/cm ² |

| | <u>Before ETO Sterilization</u> | <u>After ETO Sterilization</u> |
|------------------|-------------------------------------|------------------------------------|
| With PVB Polymer | 25 mm | 15 mm |
| Without Polymer | 26 mm | 0 |

Clearly, PVB polymer provides significant stabilization to ¹⁴C-penicillin-TDMAC against degradation caused by exposure to ethylene oxide.

Example 18

Example 1 was repeated using cellulose acetate butyrate polymer (CAB) in place of the nitrocellulose solution. The solution has the following composition:

| | |
|----------------------------------|--------|
| Cellulose Acetate Butyrate | 2.0 g |
| Ethyl acetate | 8.0 g |
| Toluene | 17.0 g |
| ¹⁴ C-penicillin-TDMAC | 2.0 g |

This solution was coated on silicone and polyurethane tubings, dried, and tested for resistance to extraction from the surface in plasma. It was also tested for resistance to degradation from ethylene oxide exposure. The following results were obtained.

| | <u>Not Extracted</u> | <u>After 24 hrs. Extraction</u> | <u>After Five Days Extraction</u> |
|---------------------|----------------------------|-------------------------------------|---------------------------------------|
| Silicone tubing | 33 μ g/cm ² | 12 μ g/cm ² | 17 μ g/cm ² |
| Polyurethane tubing | 20 μ g/cm ² | 12 μ g/cm ² | 7 μ g/cm ² |

CAB polymer clearly increases the resistance of ^{14}C -penicillin-TDMAC to extraction in plasma.

| | <u>Before ETO Sterilization</u> | <u>After ETO Sterilization</u> |
|------------------|-------------------------------------|------------------------------------|
| With CAB Polymer | 31 mm | 31 mm |
| Without Polymer | 26 mm | 0 |

This result shows how CAB polymer provides substantial stabilization to ^{14}C -penicillin-TDMAC against ETO induced degradation.

The foregoing Examples show how incorporation of these and/or other water insoluble polymers clearly improves resistance of pharmaceutical agents or pharmaceutical salts of organic ions to extraction in plasma and against the degradation effects from exposure to sterilization with ethylene oxide. At the same time, however, the incorporation of polymers still leaves effective concentrations of antibiotic or other pharmaceutical agent available at the coated surface as demonstrated by the zone of inhibition test results in Examples 16, 17 and 18.

It is expected that different polymers could be used together in a single solution/coating or in contiguous layers to further enhance performance and achieve specific results. We have also tested other polymers mixed with the pharmaceutical agents or organic ion salts of pharmaceuticals and found similarly useful improvement in resistance to extraction by plasma. These include polyisocyanates, acrylic polymers, vinylacetate, and others.

Examples 19, 20, 21 and 22 demonstrate further how various drugs can be incorporated into coatings of the invention and are suitable for use on medical devices.

Example 19

The following solution was made and coated on 1/4" ID, 1/16" wall thickness polyurethane rings and dried at 80°C.

| | |
|----------------------------|--------|
| Cellulose acetate butyrate | 0.35 g |
| Norfloxacin | 0.17 g |
| Benzylalcohol | 1.58 g |
| Toluene | 1.57 g |
| Dimethylacetamide | 5.25 g |
| Butyl Acetate | 1.58 g |

Coated rings were then extracted in artificial urine at 37°C and then tested for zone of inhibition vs. E. coli. The coating was still effective up to 35 days in the artificial urine. Coated samples sterilized by EtO were also effective against E. coli.

Example 20

2 ml. sodium methotrexate (25 mg/ml) was placed in a test tube and 4 ml. Ethanol was added. The methotrexate precipitated out of solution. Tridodecylmethylammonium chloride was added and the test tube was swirled to mix the agents. The methotrexate quickly went into solution as the tridodecylmethylammonium salt. This mixture was shaken with an equal volume of toluene to separate the water and sodium chloride from the methotrexate tridodecylmethylammonium salt. The toluene layer separated on top and had the characteristic yellow color of methotrexate salts. The aqueous layer was clear and had no color. The toluene layer was diluted with an equal volume of 2% cellulose acetate butyrate in Butyrolactone. This was coated on a polyurethane catheter surface and produced a clear layer.

Example 21

Three drops of 7.4% solution of gentamicin chloride in 62.5% water, 37.5% dimethylacetamide was diluted with 15 drops of glacial acetic acid, and 1.5 ml ethanol. Next, three drops of nitrocellulose in butyrolactone was added. This solution was clear, and produced a clear layer when coated and dried on glass.

Example 22

The following solution was coated on glass and dried for 2 minutes at 80°C.

| | |
|----------------------------|---------|
| Merbarone | 0.1 gm |
| Dimethylsulfoxide | 1.98 gm |
| Cellulose acetate butyrate | 0.12 gm |
| Ethanol | 2.0 gm |

This solution was clear, and the dried layer on glass was also clear.

Other modifications and ramifications of the present invention would appear to those skilled in the art upon a reading of this disclosure. These are intended to be included within the scope of this invention.

WHAT IS CLAIMED IS:

1. An anti-microbial composition comprising an ionic antibiotic agent reacted with an ionic organic surfactant and a water-insoluble polymer, which have been co-dissolved in a mutual solvent or a mutual solvent mixture, whereby said composition maintains high activity levels of the antibiotic over an extended period of time.
2. A pharmaceutical composition comprising an ionic pharmaceutical agent reacted with an ionic organic surfactant and a water-insoluble polymer which have been co-dissolved in a mutual solvent or a mutual solvent mixture, whereby said composition maintains high activity levels of the antibiotic over an extended period of time.
3. A pharmaceutical composition comprising a pharmaceutical agent and a water insoluble polymer which have been co-dissolved in a mutual solvent or a mutual solvent mixture, whereby said composition maintains high activity levels of the pharmaceutical agent for an extended period of time.
4. The composition in claim 1 in which the antibiotic is negatively charged and is selected from the group consisting of penicillin, ticarcillin, cefotoxin, cephalosporins, oxacillin, and carbonicillin, and the surfactant is a quaternary ammonium compound.
5. The composition in claim 1 in which the antibiotic is positively charged and the surfactant has a negatively charged organic ion such as sodium lauryl sulfate or the like.
6. The composition of claim 1 in which the antibiotic-surfactant compound is present in a concentration of about 0.5% to 99.5% by weight with the balance comprising the water-insoluble polymer.

7. The composition in claim 4 in which the surfactant is a quaternary ammonium compound selected from the group consisting of benzalkonium chloride, tridodecylmethylammonium chloride, cetylpyridinium chloride, benzyldimethylstearylammmonium chloride, and benzylcetyldimethylammonium chloride.

8. The composition in claim 1 in which the polymer is at least one selected from the group consisting of cellulose esters, polyurethane resins, acrylic polymers, condensation polymers, isocyanates, and acetals.

9. The composition of claim 1 in which the concentration of the water-insoluble polymer is about 0.01% to 40% by weight and the concentration of antimicrobial-surfactant compound is 0.01% to 40% of the coating solution.

10. The composition of claim 1 in which the water-insoluble polymer and antibiotic-surfactant compounds are in separate layers in which inter-layer molecular mingling exists at least across the interface.

11. The composition in claim 2 in which the water-insoluble polymer and pharmaceutical agent-surfactant compounds are in separate layers, in which inter-layers molecular mingling exists at least across the interface.

12. The composition in a claim 3 in which pharmaceutical agent is at least one selected from the group consisting of an antithrombogenic agent, an antibiotic agent, and an anti-cancer agent.

13. The composition in claim 3 in which the pharmaceutical agent is an antithrombogenic agent consisting of heparin complexed with an organic quaternary ammonium compound, an antibiotic agent selected from the group consisting of quinolones, aminoglycosides,

cephalosporins, sulfonamides, or tetracyclines and/or an anti-cancer agent selected from the group consisting of methotrexate or merbarone.

14. The composition in claim 3 in which the water-insoluble polymer is at least one selected from the group consisting of cellulose polymers such as cellulose nitrate, cellulose acetate, ethyl cellulose, cellulose acetatebutyrate, and cellulose acetate propionate, polyurethane resins including polyester and polyether types, acrylic polymers, condensation polymers, isocyanates, and polyacetals.

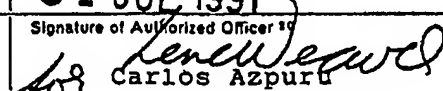
15. The composition in claim 3 in which the pharmaceutical agent is present in a concentration of about 0.5% to 99.5% by weight with the balance comprising the water-insoluble polymer.

16. The composition in claim 3 in which the water-insoluble polymer and the pharmaceutical agent are present in separate layers in which inter-layer molecular mingling exists at least across the interface, if not deeper.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US91/02868

| I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): A61K 31/74, 31/725, 37/547; A01N 37/18 U.S. CL. 424/78, 94.64 | | | | | | |
|---|--|-------------------------------------|-----------------------|------------------------|------|---|
| II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ⁴</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border: 1px solid black; padding: 5px;">Classification System</th> <th style="border: 1px solid black; padding: 5px;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; padding: 10px;">U.S.</td> <td style="border: 1px solid black; padding: 10px;">424/78, 94.64; 514/2, 12, 56 530/830; 536/21</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵</div> | | | Classification System | Classification Symbols | U.S. | 424/78, 94.64; 514/2, 12, 56 530/830; 536/21 |
| Classification System | Classification Symbols | | | | | |
| U.S. | 424/78, 94.64; 514/2, 12, 56 530/830; 536/21 | | | | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴ | | | | | | |
| Category ⁶ | Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷ | Relevant to Claim No. ¹⁸ | | | | |
| A | SCIENCE VOL. 142, "Heparin Bonding on Colloidal Graphite Surfaces", GOTT ET AL (1963), pages 1297-1298. See the Abstract. | 1-16 | | | | |
| A | Investigative Radiology, Vol. 6, July-August (1971), "A Simple Non-thrombogenic Coating" K. AMPLATZ, pages 280-289. | 1-16 | | | | |
| A | US, A, 4,871,357 (HSU ET AL) 03 OCTOBER 1989 See the Abstract. | 1-16 | | | | |
| A | Vol. XV Trans. Amer. Soc. Artif. Int. Organs (1969), GRODE ET AL. "NONTROMBOGENIC MATERIALS VIA A SIMPLE COATING PROCESS" pages 1-6. | 1-16 | | | | |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁴ Special categories of cited documents: ¹⁴</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div> | | | | | | |
| IV. CERTIFICATION | | | | | | |
| Date of the Actual Completion of the International Search ² <div style="text-align: center; font-weight: bold;">17 JUNE 1991</div> | Date of Mailing of this International Search Report ³ <div style="text-align: center; font-weight: bold; font-size: 1.2em;">31 JUL 1991</div> | | | | | |
| International Searching Authority ¹ <div style="text-align: center; font-weight: bold;">ISA/US</div> | Signature of Authorized Officer ¹⁹ <div style="text-align: center;">  for Carlos Azpur </div> | | | | | |

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.